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Anticonvulsant serotonergic and deep brain stimulation in anterior thalamus

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SUMMARY

Objective: Anterior thalamus (AN) has been shown to mediate seizures in both focal and generalized models. Specific regional increase in AN serotonergic activity was observed following AN-DBS in our pentylenetetrazol (PTZ) rodent model of acute seizures, and this increase may inhibit seizures and contribute to the mechanism of anticonvulsant DBS.

Methods: Anesthetized rats with AN-directed dialysis cannula with scalp/depth EEG were infused with PTZ at 5.5 mg/(kg min) until an EEG seizure occurred. Eight experimental groups of AN-dialysis infusion were evaluated: controls (dialysate-only), 10 and 100 μM serotonin 5-HT_7 agonist 5-carboxamidotryptamine (5-CT), 1, 10 and 100 μM serotonin antagonist methysergide (METH), AN-DBS, and 100 μM METH + AN-DBS.

Results: Latency for seizures in control animals was 3120 ± 770 s (S.D.); AN-DBS delayed onset to 5018 ± 1100 ($p < 0.01$). AN-directed 5-CT increased latency in dose-dependent fashion: 3890 ± 430 and 4247 ± 528 ($p < 0.05$). Methysergide had an unexpected protective effect at low-dose (3908 ± 550, $p < 0.05$) but not at 100 μM (2687 ± 1079). The anticonvulsant action of AN-DBS was blocked by prior dialysis using 100 μM METH. Surface EEG burst count and nonlinear analysis (H-Statistic) noted significant ($p < 0.05$) increased pre-ictal epileptiform bursts in 5-CT, methysergide, but not DBS group compared to control.

Conclusion: Increased serotonergic activity in AN raised PTZ seizure threshold, similar to DBS, but without preventing cortical bursting. 5-Carboxamidotryptamine, a 5-HT_7 agonist, demonstrated dose-dependent seizure inhibition. Methysergide proved to have an inverse, dose-dependent agonist property, antagonizing the action of AN-DBS at the highest dose. Anticonvulsant AN-DBS may in part act to selectively alter serotonin neurotransmission to raise seizure threshold.

1. Introduction

Our laboratory has in the past identified a unique cortical–subcortical pathway from mesencephalic brainstem to anterior thalamus (AN) with subsequent connections to cortex which is important in the expression of acute seizures using the chemical model pentylenetetrazol (PTZ).[1–7] We have successfully observed the efficacy of focal deep brain stimulation (DBS) in the thalamic region of this pathway as a means to raise seizure threshold.[5,8–10] Recent evidence suggests that AN may also mediate pilocarpine-induced seizures.[11] Although PTZ is a model of seizures, and not epilepsy, these data have propelled a clinical pilot trial of AN-DBS in refractory human epilepsy.[12–15] The results of this uncontrolled pilot human data of 20 patients in four North American medical centers has led to a Phase III industry-sponsored trial for refractory partial epilepsy (SANTE).[16,17]

Recently, we published data demonstrating that focal AN-serotonergic transmission may in part underscore the anticonvulsant action of site-specific AN-DBS.[18] Site and transmitter specific elevations in the serotonin metabolite 5-HIAA were observed to rise in AN microdialysis samples during both the expression of PTZ-induced acute seizures in rodents, and even more so during anticonvulsant AN-DBS, suggesting a potential link between the efficacy of DBS and serotonergic transmission. We therefore pursued electroencephalographic (EEG) evaluation of the effects of microdialysis application of serotonin agonist and antagonist compounds in the region of AN on PTZ seizures.

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2. Methods

2.1. Animal preparation

All animal studies were conducted in accordance to the Johns Hopkins Institutional Animal Care and Use Committee Guidelines. Adult male Wistar rats (200–300 g; $n = 42$), anesthetized with halothane/O_2 (1.0–2.0%) were placed in a Kopf stereotactic frame. Using aseptic surgical techniques and 1% lidocaine, the cranium was exposed and a dental drill (Foredom, Bethel, CT, USA) was used to expose the dura at coordinates derived from a stereotactic atlas[19] for bilateral placement of bipolar stimulating steel electrodes (30 kΩ, 125 μm diameter with 250 μm separation of 50 μm exposed tips) in the regions of AN (coordinates: −2.0 mm anteroposterior from bregma, 1.5 mm lateral to the sagittal suture and 5.5 mm deep from the dural edge). Depth electrode placement was followed by introduction of dialysis probe-guide cannulas in AN, and placement of four epidural EEG screw electrodes (Plastics One, Roanoke, VA, USA). Dialysis probes used in these studies with an effective dialysis membrane length of 3 mm (cut-off 6 Da, outer diameter −0.24 mm) were obtained from CMA/microdialysis (Chelmsford, MA, USA). The components were affixed as a headpiece with dental cement (Durelon, Espe, St. Paul, MN, USA). The anesthetic was discontinued and the animals were allowed to emerge from anesthesia in separate cages at room temperature.

Following a 48 h recovery period with *ad libitum* access to food and water, animals were reanesthetized with halothane (1.5%)/oxygen for less than 15 min to allow placement of cannulas into the right femoral artery and vein to monitor arterial blood pressure, arterial blood gases and for drug infusion. Thereafter, the animals were maintained at 0.5% halothane in the chamber. An approximate 85% survival rate was achieved to this point. Physiological levels of arterial blood gases were maintained throughout the experiments; rectal temperature was maintained at 37 ± 0.5 °C with a heating lamp throughout surgical procedures and the entire experimental protocol.

2.2. Serotonergic drug preparation and infusion

AN has a high population of serotonin 5-HT_7 receptors[20]; therefore we selected the relatively specific 5-HT_7 agonist 5-carboxamidotryptamine (5-CT, Sigma–Aldrich, St. Louis, MO, USA). Methysergide (METH, Sigma–Aldrich, St. Louis, MO, USA), a well-described non-selective serotonin antagonist,[21,22] was used to discern inhibition at the serotonin receptor. Microdialysis cannulas were perfused with artificial CSF (aCSF), alone at 1 μL/min, or with dissolved 5-CT or METH at appropriate concentration. The mmol/L concentration of aCSF was as follows: NaCl 131.8, $NaHCO_3$ 24.6, $CaCl_2$ 2.0, KCl 3.0, $MgCl_2$ 0.65, urea 6.7, and dextrose 3.7 in HPLC grade water. The aCSF was filtered, warmed to 37° C, and bubbled with 95% N_2/5% CO_2 until pH, O_2 and CO_2 tensions were similar to those of CSF and brain tissue.[21].

In consecutive fashion, rats were prepared as above and divided into eight treatment groups (each ≥6 animals each). Control–surgical sham with no stimulation or dialysis drug infusion; 10 μM and 100 μM 5-CT; 1 μM, 10 μM, and 100 μM METH; AN-DBS with 100 μM METH dialysis; and AN-DBS with saline AN infusion. The combination of bipolar stimulating electrode with dialysis cannula was performed with the cannula tip 1 mm dorsal to the tip of the adhered stimulating electrode. Such a construct reduced co-axial damage while permitting gravity supported diffusion. PTZ (20 mg/ml in saline, Sigma–Aldrich, St. Louis, MO, USA) was infused (T_0) beginning 20 min following initiation of dialysate infusion (T_0–20) at a rate of 5.5 mg/(kg min) via a syringe infusion pump (Harvard Instruments, Holliston, MA, USA), a rate of infusion that reliably induced seizures within 50–60 min in control animals. Following completion of experiments, animals received a lethal injection of pentobarbital and brains were perfusion-fixed with buffered 3% formalin, and brains cut for localization of electrode tips. Animals designated as AN-hit had the electrode tips in, or within 1 mm of AN.

2.3. AN stimulation

Bilateral AN-DBS was delivered using a Grass Constant Current stimulator (West Warwick, RI, USA): 100 Hz; 150 μA; 0.1 ms pulse duration beginning 40 min prior to PTZ infusion (T_0–40) and continuing during the infusion of the convulsant until the elicitation of the first severe generalized EEG seizure lasting >10 s. Stimulation current was measured by connecting the input leads from a Tektronix 5103A dual beam oscilloscope (Beaverton, OR, USA) across a 1 kΩ resistance in series with one of the stimulus output leads. The stimulation frequency of 100 Hz was selected based on our previously reported data using high frequency rate of stimulation in the anterior thalamus.[5] Continual cortical and AN EEGs were recorded using a Grass Instruments Model 79D eight-channel EEG unit. The filter settings on the EEG were 0.3 Hz (high pass filter) 300 Hz (low pass filter). The sampling rate was 1000 Hz. The epidural EEG recording was four-channel: frontal, posterior, left, right bipolar montage with electrode placement 1 cm on each side of sagittal suture, 1 cm anterior and posterior to bregma. Depth electrodes recorded regional field activity from AN.

2.4. EEG signal analysis

Seizures were defined by 10 s of rapid high voltage (>100 μV) hypersynchronous discharge (RHD). Additional paroxysms observed were a mixture of single spike (0–70 ms) or sharp (70–200 ms) activity, polysharp-polyspike, and crescendo-decrescendo 0.5–2.0 s, 50–100 μV transient bursts. All were easily discernible from background EEG. During AN-DBS trials, the amplifiers at the recording site were shut off during stimulation, and 10–15 s was allowed for dissipation of currents when switching on amplifier circuit for periodic EEG recording. EEGs from all animals were quantitatively reviewed both visually and using an H-Statistic seizure detection method.[24]

Post hoc quantification of sharp/spike count pre-ictally was performed on the 5-CT and METH infusion groups via two methods by an investigator blinded to the pharmacological or DBS stimulus effects. The first was by visual quantification of paroxysms on the digital EEG. The second was by analyzing the EEG using the H-Statistic, a nonlinear-based method which has been shown to outperform traditional spike detection methods that examine 2nd order autocorrelations out of a matched autoregressive modeled system.[25,26] The test is particularly useful in detecting nonlinear dependencies, which we have shown is characteristic of burst episodes in the recovering EEG.[27,28]

2.5. Statistical analysis

Statistical analysis was performed using multiple analysis of variance (MANOVA using both Bonferroni and Scheffe's post hoc corrections) and Student's *t*-test. Statistical software was SPSS for Windows (Version 11.0.1. 2001. Chicago: SPSS Inc.). Alpha values were set at 0.05 (two-tailed), the zero-sensitivity was therefore 0.95 for each comparison. Data are presented as mean ± S.D.

3. Results

Animals in all groups completing the experimental protocol were maintained in a stable physiological state, with an expected shift from an alkalotic to mildly acidotic state between pre-ictal to post-ictal states (pH 7.44–7.32).

3.1. Histology

Post hoc review confirmed an 81% (48/59) success rate of AN electrode/cannula insertion. Animals with electrodes or cannula in alternative locations were analyzed as a group under Section 3.2.6 (see below).

3.2. Seizure threshold

3.2.1. Controls

In control, aCSF-infused AN animals, PTZ infusion resulted in stereotypic progression of cortical EEG stimulation, progressing to a generalized seizure associated with RHD EEG discharges recorded both from AN and surface electrodes (Fig. 1). The mean onset time of the seizure was 3,120 ± 770 s (Fig. 2).

3.2.2. AN-DBS

Compared to sham control animals, bilateral AN stimulation resulted in a highly significant increase in threshold to the first generalized severe seizure which occurred at 5018 ± 1078 s after start of PTZ infusion (286 mg/kg total PTZ dose in controls vs. 460 mg/kg) ($p < 0.01$).

3.2.3. 5-CT

AN infusion of the serotonin 5-HT_7 agonist resulted in a dose-dependent delay in PTZ seizure threshold. At 10 μM, 5-CT infusion delayed seizure threshold by 25% to 3890 ± 430 s ($p < 0.05$), and at 100 μM infusion, the threshold was increased by 36% to 4247 ± 528 ($p < 0.02$) (Fig. 2).

3.2.4. Methysergide

Infusion of METH resulted in a dose-dependent response on PTZ seizure threshold. At 1 μM dialysate concentration, METH elevated seizure threshold to 3,908 ± 550, a delay by 25%. At 10 μM, PTZ seizures were delayed only to 3731 ± 1345, a < 20% increase in threshold. The downward trend prompted evaluation of 100 μM, which further lowered seizure threshold to 2687 ± 1079, statistically significant from controls ($p < 0.05$) (Fig. 2).

3.2.5. Methysergide + AN-DBS

Infusion of 100 μM METH eliminated the anticonvulsant effect of AN-DBS. The mean seizure latency of the group was virtually identical (3298 ± 1276 s) to 100 μM METH alone (3298 ± 1276) (Fig. 2).

3.2.6. Missed AN

Of the 12 animals with cannula or electrodes outside the defined boundaries of AN, two received stimulating electrode implants (AN-DBS) and the other 10 were implanted with infusion cannula for 5-CT ($n = 5$), methysergide (2), or both cannula and stimulating electrodes (3). Localization included striatum (4), medial dorsal nucleus (3), and ventral or posterior thalamic regions (5). All "AN misses" manifested electrographic seizure threshold similar to control animals, between 3075 and 3480 s.

3.3. Electroencephalographic changes

3.3.1. Direct quantitative

In controls, early, intermittent 1 s medium voltage sharp-wave bursts were observed 5–10 min following initiation of the convulsant infusion, followed by single high voltage (>150–250 μV) spike or multiple spike and sharp-wave complexes each lasting less than 1–2 s at 4–8 Hz (Fig. 3). AN-DBS paralleled the pre-ictal activity observed in control animals. Only beyond the PTZ control seizure threshold did the frequency of paroxysmal elements increase in this group of animals until seizure threshold was attained. In the serotonergic infusion studies, peri-ictal paroxysmal EEG activity was

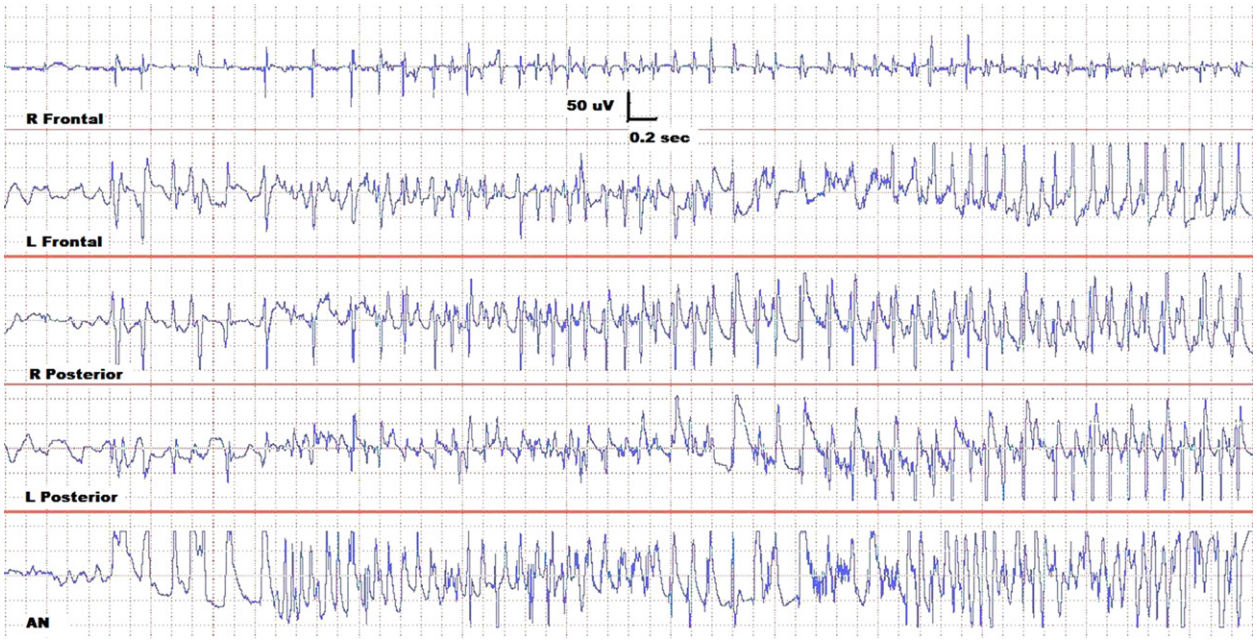

Fig. 1. Representative cortical and subcortical EEG during constant intravenous infusion of the chemical convulsant pentylenetetrazol (PTZ) in sedated animals demonstrating onset of generalized EEG seizure throughout all leads. *Abbreviations*: AN, anterior thalamus.

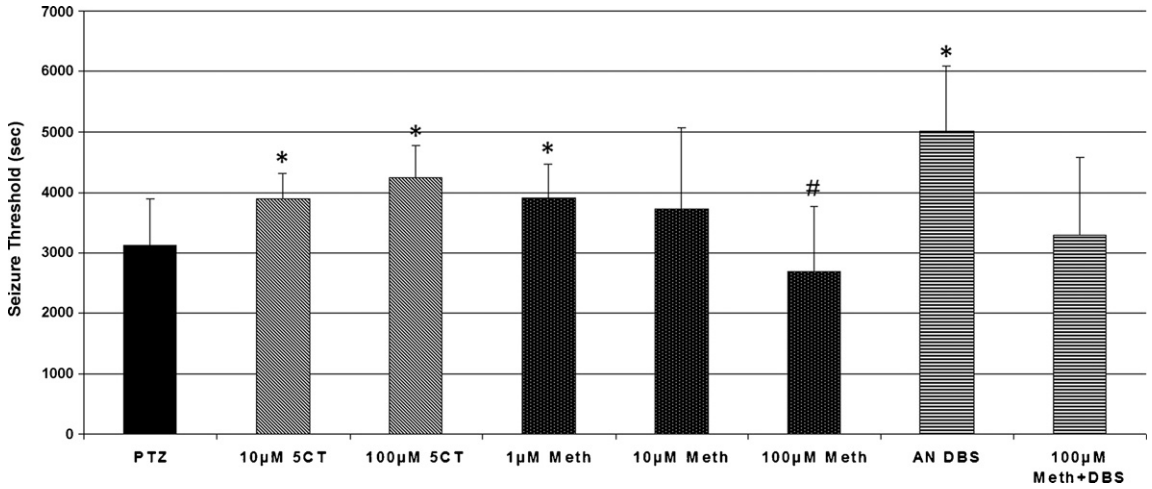

Fig. 2. Comparative seizure threshold latency to pentylenetetrazol (PTZ) between control animals (PTZ-alone) and animals treated with PTZ and AN-DBS, serotonin agonist 5-carboxamidotryptamine (5-CT), the antagonist methysergide, or methysergide + DBS. Note significant increase in latency in response to DBS and dose-dependent increasing dose of 5-CT, and the protective effect of low-dose methysergide that is lost at higher doses ± S.D., $^{**}p < 0.02$ between control (PTZ-alone) vs. either 100 μM 5-CT or AN-DBS stimulation. $^{*}p < 0.05$ control vs. 10 μM 5-CT or 1 μM methysergide, §$p < 0.05$ between AN-DBS and 100 μM methysergide and 100 μM methysergide + AN-DBS. *Abbreviations*: DBS, deep brain stimulation.

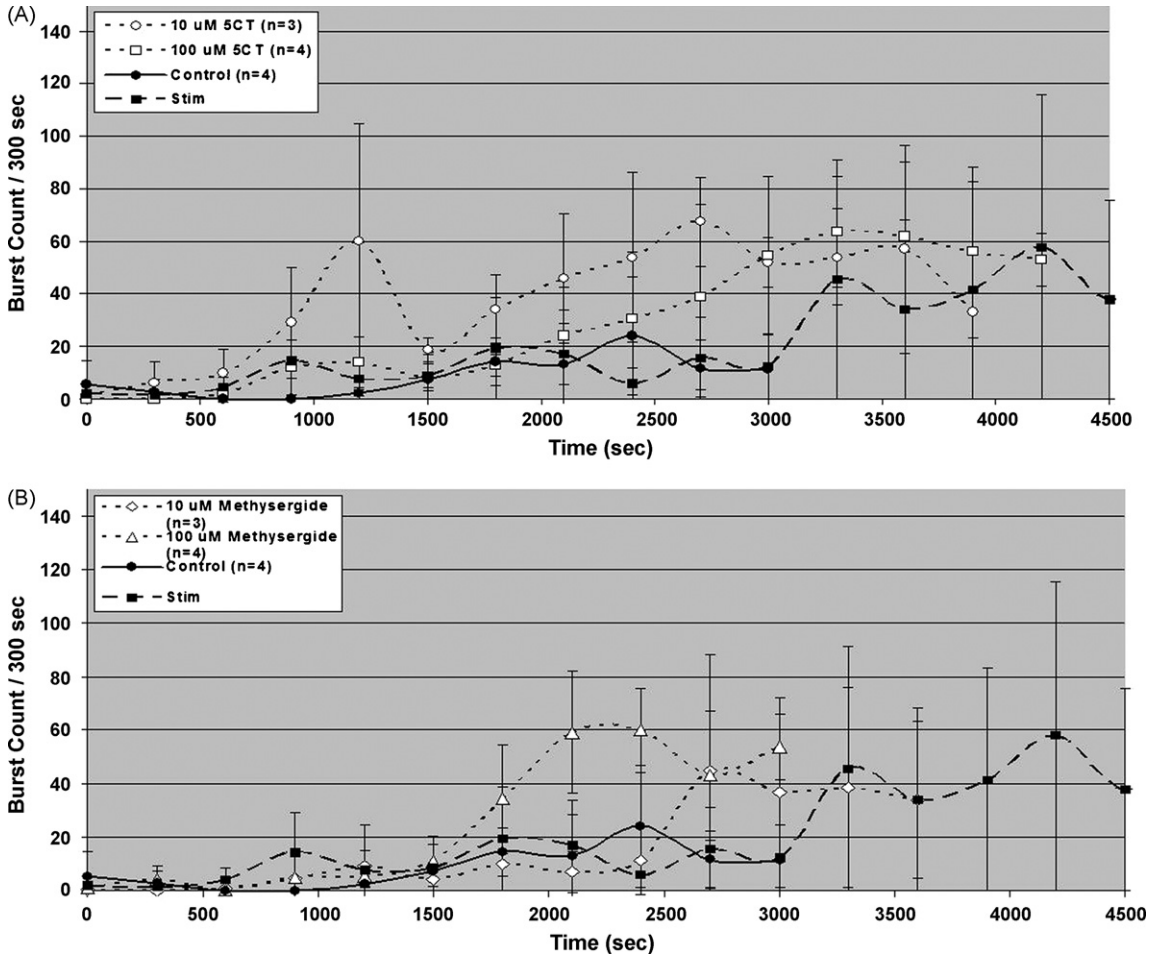

Fig. 3. Visual quantification of pre-ictal epileptiform EEG burst activity during systemic PTZ infusion. (A) Comparison between control, AN-DBS, and the serotonin agonist 5-CT at 10 μM and 100 μM AN-dialysate concentration; (B) comparison between control, AN-DBS, and the serotonin antagonist methysergide at 10 and 100 μM AN-dialysate concentration. The termination of each line represents the seizure threshold for each animal group. In both comparisons, note rise in pre-ictal paroxysms in both the 5-CT and methysergide groups as compared with AN-DBS ± S.D., $p < 0.05$ between both concentrations of 5-CT and methysergide vs. control or AN-DBS (MANOVA). *Abbreviations*: PTZ, pentylenetetrazol; AN, anterior thalamus; 5-CT, 5-carboxamidotryptamine; DBS, deep brain stimulation.

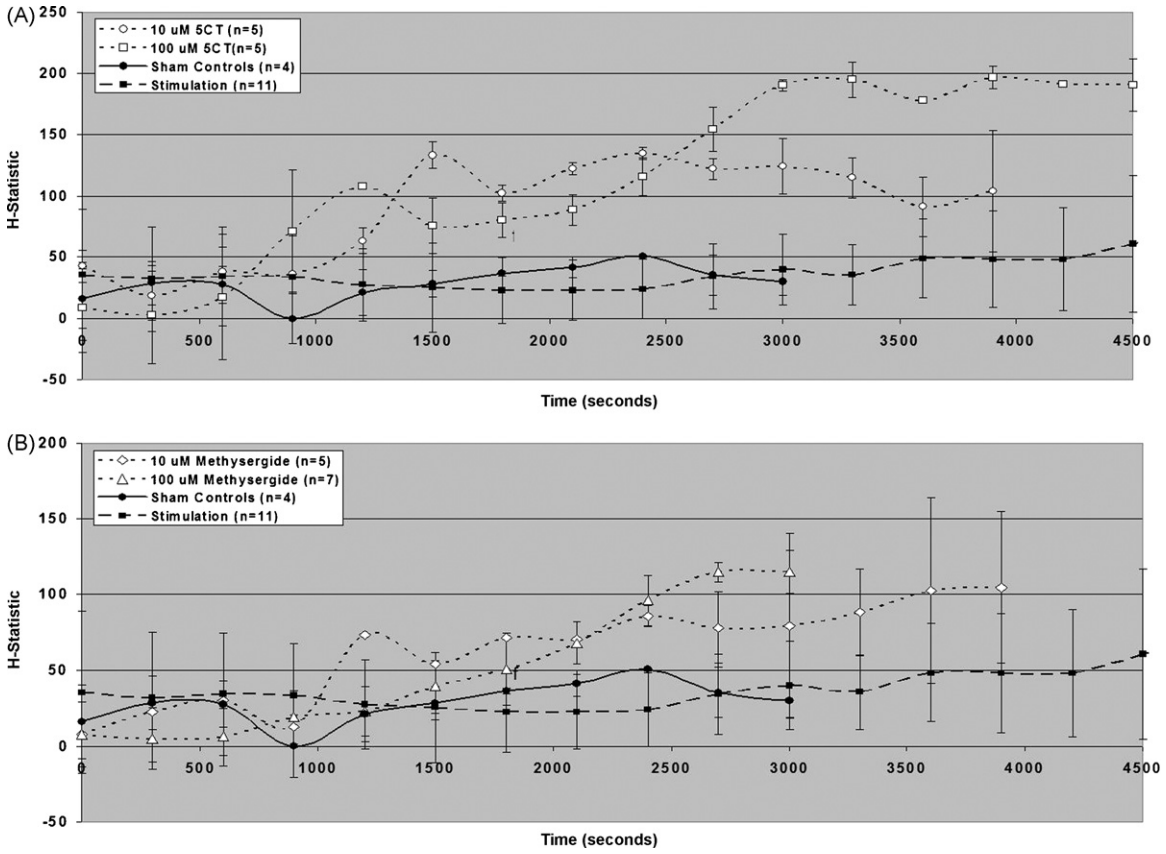

Fig. 4. H-Statistic EEG signal quantification of pre-ictal epileptiform EEG burst activity during systemic PTZ infusion. (A) Comparison between control, AN-DBS, and the serotonin agonist 5-CT at 10 and 100 μM AN-dialysate concentration; (B) comparison between control, AN-DBS, and the serotonin antagonist methysergide at 10 and 100 μM AN-dialysate concentration. Note consistent, early rise in H-Statistic (pre-2000 s) in the 5-CT and methysergide groups as compared to visual inspection data in Fig. 3. ±S.D., $p < 0.05$ between both concentrations of 5-CT and methysergide vs. control or AN-DBS (MANOVA); $p < 0.05$ between H-Statistic and visual inspection in 5-CT and methysergide groups. *Abbreviations*: PTZ, pentylenetetrazol; AN, anterior thalamus; 5-CT, 5-carboxamidotryptamine; DBS, deep brain stimulation.

consistently greater in both the 5-CT and METH groups than in controls ($p < 0.05$), despite a robust anticonvulsant effect by 5-CT and low-dose METH in raising seizure threshold (Fig. 3). Even during high-dose METH infusion that resulted in control level seizure threshold, the pre-ictal spike and sharp-wave activity was much greater than in the control population.

3.3.2. H-Statistic

The H-Statistic was employed to quantify all epileptiform paroxysms, both subtle and overt. In comparison between visual inspection and the H-Statistic in both the transmitters and DBS interventions (Fig. 4), the latter was particularly more sensitive earlier in the PTZ infusion (pre-2000 s), where subtle, yet consistent epileptiform aberrations of the EEG failed to be discriminated from background by simple visual inspection ($p < 0.05$ between two methods).

4. Discussion

It was observed in previous experiments that anticonvulsant stimulation specifically raised serotonergic turnover in AN as compared to other thalamic transmitter systems, and suggested that AN-DBS may impart its anticonvulsant action against systemic PTZ through alteration of AN serotonergic activity.[18] The results here clearly demonstrated that serotonergic agonist modulation using 5-CT within AN did indeed raise seizure threshold in a dose-dependent fashion. This anticonvulsant effect, using the higher of the two doses (100 μM), paralleled the elevation in seizure threshold induced by focal AN-DBS. High-dose METH, in contrast, antagonized the action of AN-DBS. In addition, blocking the anticonvulsant effect of AN-DBS by the serotonin antagonist METH supports the hypothesis that DBS may therapeutically act, in part, via alteration of transmitter mediated synaptic function.

The implication that serotonergic transmission may play a key role in seizure expression has also been well recognized for some time,[29–33] and demonstrated in both chemical seizure models as well as genetic epilepsy-prone rodents (GEPR).[34–37] It has been hypothesized in GEPR rats, for example, that there is a deficit of regional brain serotonin that leads to seizure susceptibility,[38,39] and such reasoning is supported by the efficacy in treatment observed with serotonin re-uptake inhibitors.[37] Agents that antagonize serotonin binding conversely have a tendency to act as proconvulsants.[35,37,39,40]

What remains obscure is the precise relationship this transmitter plays within pro- and anticonvulsant networks since experimental studies are often contradictory. In our model, 5-HT_7 agonism by 5-CT raised seizure threshold to PTZ, but blocking this receptor subtype was protective in a mouse audiogenic seizure model.[38] Such ambiguity may reside in evidence that points to both somatodendritic as well as postsynaptic 5-HT receptors, thus enabling a variety of facilitory and inhibitory actions in different model systems.[41,42] Even within a given seizure model, differential affinities of 5-HT agonist and antagonist agents for pre- vs. postsynaptic binding sites (such as the receptors 5-$HT1_B$ and 5-HT_{1A}, respectively) may lead to opposite actions regarding proconvulsant tendencies.

In our study, 5-CT was used as a high-affinity agonist at the 5-HT_7 receptor, highly concentrated in AN in rodents.[20,22] METH is a well described non-selective serotonin antagonist,[21,23,42–44], and has been reported to specifically exacerbate PTZ seizures in mice.[31] Nonetheless, in our seizure model, low-dose METH increased seizure threshold, and only at higher doses was specific antagonism of 5-CT observed. It is likely that the non-selective property of METH underscores its variable action. Similar observations were made by Holden et al. where METH inexplicably proved no different from control in antagonizing serotonin 5-HT_1 and 5-HT_3 receptor effects.[45] The authors suggested that the broad action of METH against serotonin receptors may underlie the poor response.

The mechanisms of DBS, whether for epilepsy or movement disorders, also remain incompletely understood. There is some evidence that stimulation may result in a predominant inhibitory influence through membrane hyperpolarization.[46] Using a finite element model of a DBS electrode with the field applied to a three-dimensional multi-compartment neuron model, evidence supports that the stimulation effects were excitatory near the electrode and that synaptic terminals and axons of passage had lower thresholds and were excited over a greater volume of tissue than local neuronal cell bodies.[47,48] The synaptic terminal may also be independently influenced by DBS. Such threshold data may explain how output can be increased from a stimulated structure even though local neuronal activity is inhibited.[49,50] The presence of dorsal raphe nucleus (DRN) to AN serotonergic pathways and 5-HT_1 heteroreceptors mapped to terminals of AN-derived thalamocortical projections[51] supports the possibility of such serotonergic seizure modulation within AN.

The greater anticonvulsant effect of AN DBS than dialyzed 5-CT may reflect a more localized effect of electrical stimulation compared to the diffusion effects of dialyzed serotonin agonists. The current–distance curve presented by Ranck indicates that a 0.2 ms 150 μA pulse from a monopolar depth electrode stimulates axons or cell bodies only up to 1.0 mm away.[49] In contrast, autoradiographic studies using dialysis probes similar to ours demonstrated that label spread radially from the dialysis probe with a maximum diameter of approximately 3 mm by 1 h of perfusion.[52] Alternatively, as electrical stimulation preferentially activates fibers of passage rather than neurons, the AN-DBS may affect different neuronal networks that contribute to the final thalamocortical influence on the cortical EEG. Such distinction between the effects of dialysis and DBS appears corroborated by different actions on the pre-ictal cortical EEG. As noted by the H-Statistic signal analysis, electrical stimulation during the DBS pre-ictal period greatly reduced the seizure paroxysms as compared to the pre-ictal period when 5-CT or METH was administered. AN-DBS appeared to delay the seizure while not altering the direct cortical action of PTZ, while both 5-CT and METH gave rise to large incremental increases in the pre-ictal H-Statistic.

How these data may translate to human epilepsy remains conjectural. Although PTZ has been used for decades as an effective screening tool for clinical anticonvulsants, the chemical is an animal model of acute seizures, not epilepsy. Results of current AN-DBS in human epilepsy studies may offer clinical correlation. These current data do, however, support the premise that electrical stimulation may act in part via modulating serotonergic activity. Our results also distinguish between DBS and serotonin mechanisms in raising seizure threshold, as evidenced by distinct influences on the pre-ictal cortical EEG. Further investigation using selective 5-HT_7 antagonists and with concurrent AN-DBS would prove useful in further discriminating the anticonvulsant actions of these two methods.

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Conflict of interest

None.

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